REMARKS

I. Status of the Application

Claims 10 and 13-28 are pending in the application. Applicants gratefully acknowledge the Examiner's withdrawal of her rejections of claims 20-28 under 35 § U.S.C. 102(b) as anticipated by Gronthos et al. Claims 10 and 13-19 remain rejected under 35 § U.S.C. 112, first paragraph. Claims 10, 13-16, 18, 20, 22 and 23 stand rejected under 35 § U.S.C. 102(b) as anticipated by Gazit et al., (1989) *Connective Tissue Research* 23:153. Claims 25, 26 and 28 stand rejected under 35 § U.S.C. 102(b) as anticipated by Gleave et al. (1992) *The Journal of Neurology* 147:1151.

Applicants also acknowledge that claims 21, 24 and 27 have been objected to presumably as being dependent upon a rejected base claim, but would be allowable if rewritten into independent form including all of the limitations of the base claim.

In response, Applicants have amended the independent claims 10, 20 and 25 to include the language of claims 24 and 27 insofar as to recite inducing cells to differentiate by one or more inductors of differentiation.

Applicants respectfully request entry and consideration of the foregoing amendments, which are intended to place this case in condition for allowance.

II. The Specification Provides Adequate Written Description for Claims 10 and 13-19

At page 3, paragraph 1 of the instant Office Action, claims 10 and 13-19 remain rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter that was not described in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The Examiner is of the

opinion that the pages Applicants have pointed out do not teach the steps recited in claim 10. The Examiner concludes that the scientific reasoning and evidence as a whole indicates that the rejection should be maintained.

Applicants traverse this rejection. Applicants respectfully submit that the specification provides adequate support for the limitations of claim 10 and claims depending therefrom. Claim 10 recites: **[1]** a method of producing active factors comprising the steps of: **[2]** (a) applying undifferentiated mammalian cells on a substrate; **[3]** (b) contacting the cells with a culture medium for a sufficient time to produce a matrix; **[4]** (c) contacting the cells with the culture medium for a sufficient time to produce active factors; **[5]** (d) removing the substrate and the matrix from the culture medium; and **[6]** (e) recovering the active factors from the culture medium.

Support for **[1]** "a method of producing active factors," can be found at least at page 4, line 4 of the specification, where Applicants teach "a method of producing active factors." Support for **[2]** "applying undifferentiated mammalian cells on a substrate," can be found at least at page 4, lines 6-7 of the specification, where Applicants teach "applying undifferentiated mammalian cells...on a substrate." Support for **[3]** "contacting the cells with a culture medium for a sufficient time to produce a matrix," can be found at least at page 2, lines 3-4 of the specification, where Applicants teach "contacting said cells with a culture medium for a sufficient time to produce a mineralised or non-mineralised matrix." Support for **[4]** "contacting the cells with the culture medium for a sufficient time to produce active factors," can be found at least at page 4, lines 8-9 of the specification, where Applicants teach "contacting said cells with a culture medium for a sufficient time to produce growth factors," and at page 4, lines 15 of the specification, where Applicants teach that active factors comprise growth factors. Support for

[5] "removing the substrate and the matrix from the culture medium," can be found at least at page 4, line 10 of the specification, where Applicants teach "removing the substrate with the matrix from the culture medium." Support for **[6]** "recovering the active factors from the culture medium" can be found at least at page 4, lines 11 of the specification, where Applicants teach "recovering the active factors from the culture medium."

Applicants' abstract teaches that the culture medium from a method for *in vitro* production of bone tissue can also be used for the production of active factors (lines 1-2 and 11-12). Therefore, Applicants' disclosure of methods for producing bone tissue are applicable to Applicants' methods of producing active factors.

Thus, one of skill in the art would recognize in Applicants' disclosure a description of the invention defined by claim 10 and the claims depending therefrom. Accordingly, Applicants respectfully request that this rejection be reconsidered and withdrawn.

III. The Rejections Based On Gazit et al. And Gleave et al.

At page 4, paragraph 2 of the instant Office Action, claims 10, 13-16, 18, 20, 22 and 23 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Gazit et al., (1994) *Connective Tissue Research* 23:153. At page 5, paragraph 1 of the instant Office Action, claims 25, 26 and 28 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Gleave et al., (1994) *The Journal of Urology* 147:1151.

In response, Applicants have included the limitation of claims 24 and 27 to independent claims 10, 20 and 25 insofar as to recite inducing cells to differentiate by one or more inductors of differentiation. Applicants respectfully submit that the claims as amended distinguish over Gazit et al. and Gleave et al.

Gazit et al. is directed to characterizing growth-promoting components during bone marrow regeneration. Gazit et al. neither teaches nor suggests applying undifferentiated cells on a substrate and inducing the cells to differentiate by one or more inductors of differentiation. Instead, Gazit teaches

> "[c]onditioned medium was prepared from healing marrow as described previously. Briefly, tissue was separated from the marrow space of rat tibiae 10 days after ablation and incubated for ***24 h*** in ***serum-free*** F-10 medium supplemented with 1% (vol./vol.) penicillin-streptomycin at 37° in 5% CO_2-air. The medium was the collected..." (page 154, 3d paragraph, emphasis added).

Serum-free F-10 medium contains only inorganic salts, amino acids, vitamins, D-glucose, hypoxyanthine sodium, lipoic acid, phenol red, sodium pyruvate and thymidine (see Attachment A, F-10 product information from Invitrogen). Factors necessary for differentiation of the bone marrow cells *in vitro* are not present.

Gleave et al. is directed to characterizing the ability of different fibroblasts to stimulate the growth of a human prostate cancer cell line. Gleave et al. teaches generating fibroblast conditioned media by growing prostate-derived and bone-derived fibroblasts for 48 hours and collecting the media. Gleave et al. neither teaches nor suggests contacting stromal cells with a culture medium and inducing differentiation by one or more inductors of differentiation. Gleave et al. is completely silent regarding the induction of differentiation using exogenous factors such as glucocorticoids.

Accordingly, Applicants respectfully request that the rejections based on Gazit et al. and Gleave et al. under 35 U.S.C. § 102(b) be reconsidered and withdrawn.

IV. Conclusion

Having responded to all outstanding issues, reconsideration and allowance of all the pending claims is respectfully requested. If a telephone conversation with Applicants' attorney would expedite prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 720-9600.

Respectfully submitted,

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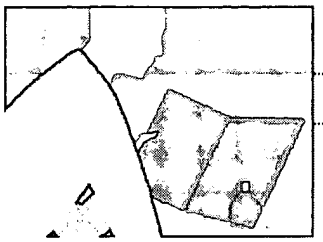
John P. Iwanicki, Reg. No. 34,628
BANNER & WITCOFF, LTD.
28 State Street, 28th Floor
Boston, MA 02109
(617) 720-9600



Technical Resources

- Brochures and Product Info
- Cell Line Database
- Certificate of Analysis
- Citations
- Contact Technical Services
- Expression Wizard
- FAQs
- Limited Use Label Licenses
- Manuals and Product Inserts
- Media Formulations
- MSDS
- Newsletters and Journals
- Online Seminars
- Protocols
- Transfection Collection
- Vector Data

Technical Resources - Media Formulations

F-10 Nutrient Mixure (Ham) (1X), liquid (11550)
Contains L-glutamine

Ham's Nutrient Mixtures were originally developed to support the clonal growth of CHO cells as well as mouse L-cells. Ham's F-10 has been shown to support the the growth of human diploid cells, WBC an explants of rat rabbit and chicken.

Catalogue Number: 11550

COMPONENTS	Mole. Weight	Conc. (mg/L)	Molarity (mM)
INORGANIC SALTS			
Calcium chloride (CaCl2)	111	33.30	0.30
Cupric sulfate (CuSO4-5H2O)	250	0.0025	0.00001
Ferrous sulfate (FeSO4-7H2O)	278	0.834	0.003
Potassium chloride (KCl)	75	285.00	3.80
Potassium phosphate, mono. (KH2PO4)	136	83.00	0.61
Magnesium sulfate (MgSO4)	120	74.62	0.622
Sodium chloride (NaCl)	58	7400.00	127.00
Sodium bicarbonate (NaHCO3)	84	1200.00	14.30
Sodium phosphate, dibas. (Na2HPO4)	142	153.70	1.08
Zinc sulfate (ZnSO4-7H2O)	288	0.03	0.0001
OTHER COMPONENTS			
D-Glucose	180	1100.00	6.10
Hypoxanthine Na	159	4.70	0.0296
Lipoic Acid	206	0.20	0.00097
Phenol Red	398	1.20	0.003
Sodium Pyruvate	110	110.00	1.00
Thymidine	242	0.70	0.0029
AMINO ACIDS			
L-Alanine	89	9.00	0.101
L-Arginine HCl	211	211.00	1.00
L-Asparagine H2O	150	15.00	0.100
L-Aspartic acid	133	13.00	0.098
L-Cysteine	121	25.00	0.207
L-Glutamic acid	147	14.70	0.100
L-Glutamine	146	146.00	1.00
Glycine	75	7.50	0.100
L-Histidine HCl H2O	210	23.00	0.110
L-Isoleucine	131	2.60	0.0198

L-Leucine	131	13.00	0.0992
L-Lysine hydrochloride	183	29.00	0.158
L-Methionine	149	4.50	0.030
L-Phenylalanine	165	5.00	0.0303
L-Proline	115	11.50	0.100
L-Serine	105	10.50	0.100
L-Threonine	119	3.60	0.030
L-Tryptophan	204	0.60	0.0029
L-Tyrosine 2Na 2H2O	261	2.62	0.010
L-Valine	117	3.50	0.0299
VITAMINS			
Biotin	244	0.024	0.000098
D-Calcium pantothenate	477	0.70	0.0014
Choline chloride	140	0.70	0.005
Folic acid	441	1.30	0.0029
i-Inositol	180	0.50	0.003
Niacinamide	122	0.60	0.005
Pyridoxine HCl	206	0.20	0.001
Riboflavin	376	0.40	0.001
Thiamine hydrochloride	337	1.00	0.0029
Vitamin B12	1,355	1.40	0.001

REFERENCE:

1. Ham, R.G., (1963) An improved nutrient solution for diploid Chinese hamster and human cell l Res., 29:515.